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	L #	Hits	Search Text	DBs	Time Stamp
1	L1	354	(alpha adj amylase\$1) same bacillus same (mutant\$1 or variant\$1)	USPAT; US-PGPUB	2003/06/24 09:22
2	L2	5575	(mutant\$1 or variant\$1) same (stability or thermostability or calcium adj depend\$8)	USPAT; US-PGPUB	2003/06/24 09:29
3	L3	198	1 and 2	USPAT; US-PGPUB	2003/06/24 09:29

\* \* \* \* \* STN Columbus \* \* \* \* \*

FILE 'HOME' ENTERED AT 13:51:52 ON 24 JUN 2003

=> fil .bec

COST IN U.S. DOLLARS

SINCE FILE	TOTAL
ENTRY	SESSION

FULL ESTIMATED COST

0.21	0.21
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FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS, ESBIODASE, BIOTECHNO, WPIDS' ENTERED AT 13:52:13 ON 24 JUN 2003  
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s alpha amylase#

FILE 'MEDLINE'

447353 ALPHA

19952 AMYLASE#

L1 4399 ALPHA AMYLASE#

(ALPHA (W) AMYLASE#)

FILE 'SCISEARCH'

630747 ALPHA

15981 AMYLASE#

L2 7110 ALPHA AMYLASE#

(ALPHA (W) AMYLASE#)

FILE 'LIFESCI'

145006 "ALPHA"

4245 AMYLASE#

L3 2543 ALPHA AMYLASE#

("ALPHA" (W) AMYLASE#)

FILE 'BIOTECHDS'

23121 ALPHA

4710 AMYLASE#

L4 3163 ALPHA AMYLASE#

(ALPHA (W) AMYLASE#)

FILE 'BIOSIS'

587478 ALPHA

26799 AMYLASE#

L5 9525 ALPHA AMYLASE#

(ALPHA (W) AMYLASE#)

FILE 'EMBASE'

499172 "ALPHA"

14617 AMYLASE#

L6 3240 ALPHA AMYLASE#

("ALPHA" (W) AMYLASE#)

FILE 'HCAPLUS'

1408155 ALPHA

42248 AMYLASE#

L7 17466 ALPHA AMYLASE#

(ALPHA (W) AMYLASE#)

FILE 'NTIS'

28393 ALPHA

163 AMYLASE#

L8 60 ALPHA AMYLASE#

(ALPHA (W) AMYLASE#)

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FILE 'ESBIOBASE'
    171445 ALPHA
    3652 AMYLASE#
L9      1737 ALPHA AMYLASE#
        (ALPHA (W) AMYLASE#)

FILE 'BIOTECHNO'
    177086 ALPHA
    4026 AMYLASE#
L10     2049 ALPHA AMYLASE#
        (ALPHA (W) AMYLASE#)

FILE 'WPIDS'
    162271 ALPHA
    5020 AMYLASE#
L11     2076 ALPHA AMYLASE#
        (ALPHA (W) AMYLASE#)

TOTAL FOR ALL FILES
L12     53368 ALPHA AMYLASE#

=> s l12(3a)(muta? or variant#)
FILE 'MEDLINE'
    409187 MUTA?
    89068 VARIANT#
L13     57 L1 (3A) (MUTA? OR VARIANT#)

FILE 'SCISEARCH'
    386646 MUTA?
    93552 VARIANT#
L14     72 L2 (3A) (MUTA? OR VARIANT#)

FILE 'LIFESCI'
    185562 MUTA?
    30766 VARIANT#
L15     54 L3 (3A) (MUTA? OR VARIANT#)

FILE 'BIOTECHDS'
    34388 MUTA?
    10241 VARIANT#
L16     116 L4 (3A) (MUTA? OR VARIANT#)

FILE 'BIOSIS'
    456180 MUTA?
    93859 VARIANT#
L17     157 L5 (3A) (MUTA? OR VARIANT#)

FILE 'EMBASE'
    332869 MUTA?
    77197 VARIANT#
L18     50 L6 (3A) (MUTA? OR VARIANT#)

FILE 'HCAPLUS'
    418917 MUTA?
    86673 VARIANT#
L19     265 L7 (3A) (MUTA? OR VARIANT#)

FILE 'NTIS'
    9391 MUTA?
    4387 VARIANT#
L20     0 L8 (3A) (MUTA? OR VARIANT#)

FILE 'ESBIOBASE'
    189272 MUTA?

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      32332 VARIANT#
L21      30 L9 (3A) (MUTA? OR VARIANT#)

FILE 'BIOTECHNO'
      226128 MUTA?
      38635 VARIANT#
L22      42 L10 (3A) (MUTA? OR VARIANT#)

FILE 'WPIDS'
      21493 MUTA?
      20668 VARIANT#
L23      58 L11 (3A) (MUTA? OR VARIANT#)

TOTAL FOR ALL FILES
L24      901 L12 (3A) (MUTA? OR VARIANT#)

=> s l12(3a) (Bacillus or termamyl)
FILE 'MEDLINE'
      43147 BACILLUS
      8 TERMAMYL
L25      415 L1 (3A) (BACILLUS OR TERMAMYL)

FILE 'SCISEARCH'
      42526 BACILLUS
      42 TERMAMYL
L26      623 L2 (3A) (BACILLUS OR TERMAMYL)

FILE 'LIFESCI'
      23044 BACILLUS
      12 TERMAMYL
L27      426 L3 (3A) (BACILLUS OR TERMAMYL)

FILE 'BIOTECHDS'
      15368 BACILLUS
      52 TERMAMYL
L28      907 L4 (3A) (BACILLUS OR TERMAMYL)

FILE 'BIOSIS'
      62404 BACILLUS
      63 TERMAMYL
L29      926 L5 (3A) (BACILLUS OR TERMAMYL)

FILE 'EMBASE'
      31699 BACILLUS
      24 TERMAMYL
L30      409 L6 (3A) (BACILLUS OR TERMAMYL)

FILE 'HCAPLUS'
      74546 BACILLUS
      318 TERMAMYL
L31      1972 L7 (3A) (BACILLUS OR TERMAMYL)

FILE 'NTIS'
      1613 BACILLUS
      0 TERMAMYL
L32      4 L8 (3A) (BACILLUS OR TERMAMYL)

FILE 'ESBIOBASE'
      12152 BACILLUS
      15 TERMAMYL
L33      199 L9 (3A) (BACILLUS OR TERMAMYL)

FILE 'BIOTECHNO'
      19004 BACILLUS

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17 TERMAMYL  
L34 371 L10(3A) (BACILLUS OR TERMAMYL)

FILE 'WPIDS'  
10642 BACILLUS  
34 TERMAMYL  
L35 178 L11(3A) (BACILLUS OR TERMAMYL)

TOTAL FOR ALL FILES  
L36 6430 L12(3A) (BACILLUS OR TERMAMYL)

=> s l24 and l36  
FILE 'MEDLINE'  
L37 20 L13 AND L25

FILE 'SCISEARCH'  
L38 22 L14 AND L26

FILE 'LIFESCI'  
L39 17 L15 AND L27

FILE 'BIOTECHDS'  
L40 57 L16 AND L28

FILE 'BIOSIS'  
L41 45 L17 AND L29

FILE 'EMBASE'  
L42 17 L18 AND L30

FILE 'HCAPLUS'  
L43 120 L19 AND L31

FILE 'NTIS'  
L44 0 L20 AND L32

FILE 'ESBIOBASE'  
L45 5 L21 AND L33

FILE 'BIOTECHNO'  
L46 10 L22 AND L34

FILE 'WPIDS'  
L47 29 L23 AND L35

TOTAL FOR ALL FILES  
L48 342 L24 AND L36

=> s l48 not 1997-1999/py  
FILE 'MEDLINE'  
1333854 1997-1999/PY  
L49 18 L37 NOT 1997-1999/PY

FILE 'SCISEARCH'  
2862496 1997-1999/PY  
L50 20 L38 NOT 1997-1999/PY

FILE 'LIFESCI'  
337241 1997-1999/PY  
L51 15 L39 NOT 1997-1999/PY

FILE 'BIOTECHDS'  
41041 1997-1999/PY  
L52 44 L40 NOT 1997-1999/PY

FILE 'BIOSIS'  
1680802 1997-1999/PY  
L53 41 L41 NOT 1997-1999/PY

FILE 'EMBASE'  
1252969 1997-1999/PY  
L54 15 L42 NOT 1997-1999/PY

FILE 'HCAPLUS'  
2535600 1997-1999/PY  
L55 86 L43 NOT 1997-1999/PY

FILE 'NTIS'  
85443 1997-1999/PY  
L56 0 L44 NOT 1997-1999/PY

FILE 'ESBIOBASE'  
831730 1997-1999/PY  
L57 3 L45 NOT 1997-1999/PY

FILE 'BIOTECHNO'  
338670 1997-1999/PY  
L58 9 L46 NOT 1997-1999/PY

FILE 'WPIDS'  
2351668 1997-1999/PY  
L59 12 L47 NOT 1997-1999/PY

TOTAL FOR ALL FILES  
L60 263 L48 NOT 1997-1999/PY

=> s 160 not 2000-2003/py  
FILE 'MEDLINE'  
1753010 2000-2003/PY  
L61 17 L49 NOT 2000-2003/PY

FILE 'SCISEARCH'  
3309811 2000-2003/PY  
L62 19 L50 NOT 2000-2003/PY

FILE 'LIFESCI'  
336333 2000-2003/PY  
L63 14 L51 NOT 2000-2003/PY

FILE 'BIOTECHDS'  
61757 2000-2003/PY  
L64 34 L52 NOT 2000-2003/PY

FILE 'BIOSIS'  
1803382 2000-2003/PY  
L65 30 L53 NOT 2000-2003/PY

FILE 'EMBASE'  
1500007 2000-2003/PY  
L66 15 L54 NOT 2000-2003/PY

FILE 'HCAPLUS'  
3330626 2000-2003/PY  
L67 64 L55 NOT 2000-2003/PY

FILE 'NTIS'  
55117 2000-2003/PY  
L68 0 L56 NOT 2000-2003/PY

FILE 'ESBIOBASE'  
967524 2000-2003/PY  
L69 3 L57 NOT 2000-2003/PY

FILE 'BIOTECHNO'  
395493 2000-2003/PY  
L70 9 L58 NOT 2000-2003/PY

FILE 'WPIDS'  
2981530 2000-2003/PY  
L71 1 L59 NOT 2000-2003/PY

TOTAL FOR ALL FILES  
L72 206 L60 NOT 2000-2003/PY

=> dup rem l72  
PROCESSING COMPLETED FOR L72  
L73 108 DUP REM L72 (98 DUPLICATES REMOVED)

=> d tot

L73 ANSWER 1 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI Laundry detergent containing a **mutant alpha-amylase**;  
Bacillus licheniformis recombinant enzyme production by enzyme  
engineering, for application as a laundry surfactant  
AU Barnett C C; Boyer S G; Mitchinson C; Power S D  
AN 1997-00549 BIOTECHDS  
PI WO 9630481 3 Oct 1996

L73 ANSWER 2 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI New **alpha-amylase variants**;  
mutant enzyme construction for improved calcium dependency, substrate  
binding, cleavage, pH dependent activity and thermostability;  
application in e.g. surfactant composition  
AU Svendsen A; Bisgard-Frantzen H; Borchert T V  
AN 1996-12567 BIOTECHDS  
PI WO 9623874 8 Aug 1996

L73 ANSWER 3 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI Improved bleach-containing cleaning composition;  
granular bleach-containing granular surfactant containing  
**Bacillus licheniformis mutant alpha-amylase** and **Bacillus amyloliquefaciens**  
**mutant protease**  
AU Barnett C C; Mitchinson C; Power S D  
AN 1996-06019 BIOTECHDS  
PI WO 9605295 22 Feb 1996

L73 ANSWER 4 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI Acid-stable and thermostable alpha-amylase gene;  
characterization from Bacillus licheniformis; enzyme engineering  
AN 1997-01693 BIOTECHDS  
PI JP 08289788 5 Nov 1996

L73 ANSWER 5 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
TI Purification and characterization of a truncated **Bacillus**  
**subtilis .alpha.-amylase** produced by Escherichia coli  
SO Applied Microbiology and Biotechnology (1996), 44(6), 746-52  
CODEN: AMBIDG; ISSN: 0175-7598  
AU Marco, J. L.; Bataus, L. A.; Valencia. F. F.; Ulhoa, C. J.; Astolfi-Filho,  
S.; Felix, C. R.  
AN 1996:206488 HCAPLUS



DN 124:336337

L73 ANSWER 6 OF 108 HCAPLUS COPYRIGHT 2003 ACS

TI Structure-function studies of two polysaccharide-degrading enzymes:

**Bacillus** stearothermophilus **.alpha.-amylase**  
and *Trichoderma reesei* cellobiohydrolase II

SO VTT Publications (1996), 277, 143pp

CODEN: VTTPEY; ISSN: 1235-0621

AU Koivula, Anu

AN 1996:749573 HCAPLUS

DN 126:28530

L73 ANSWER 7 OF 108 HCAPLUS COPYRIGHT 2003 ACS

TI Instability of **.alpha.-amylase** production and morphological variation in continuous culture of *Bacillus amyloliquefaciens* is associated with plasmid loss

SO Process Biochemistry (Oxford) (1996), Volume Date 1997, 32(1), 51-59

CODEN: PBCHE5; ISSN: 1359-5113

AU Hillier, P.; Wase, D. A. J.; Emery, A. N.; Solomons, G. L.

AN 1996:695106 HCAPLUS

DN 125:326485

L73 ANSWER 8 OF 108 HCAPLUS COPYRIGHT 2003 ACS

TI Hyperthermostable mutants of *Bacillus licheniformis*: thermodynamic studies and structural interpretation

SO Perspectives on Protein Engineering '96, [International Conference], 5th, Montpellier, Fr., 1996 (1996), Paper No. 7, 9 pp.. Editor(s): Geisow, Michael J. Publisher: BIODIGM, Bingham, UK.

CODEN: 64HIAR

AU Declerck, Nathalie; Gaillardin, Claude; Machius, Mischa; Wiegand, Georg; Huber, Robert

AN 1997:287296 HCAPLUS

DN 126:314064

L73 ANSWER 9 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

TI **Mutant** *B. licheniformis* **alpha-amylase** enzymes;

*Bacillus licheniformis* mutant thermostable enzyme production; application in starch degradation, textile or paper desizing, brewing industry and as household surfactant

AU van der Laan J M; Aehle W

AN 1996-03039 BIOTECHDS

PI WO 9535382 28 Dec 1995

L73 ANSWER 10 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

TI New **alpha-amylase variants**;

**Bacillus liquefaciens alpha-amylase**

enzyme engineering for improved thermostability, pH stability, etc.; application in surfactant composition to improve washing performance

AU Bisgard-Frantzen H; Borchert T V; Svendsen A; Thellersen M; van der Zee P

AN 1995-07973 BIOTECHDS

PI WO 9510603 20 Apr 1995

L73 ANSWER 11 OF 108 MEDLINE DUPLICATE 1

TI Hyperthermostable **mutants** of *Bacillus licheniformis* **alpha-amylase**: multiple amino acid replacements and molecular modelling.

SO PROTEIN ENGINEERING, (1995 Oct) 8 (10) 1029-37.

Journal code: 8801484. ISSN: 0269-2139.

AU Declerck N; Joyet P; Trosset J Y; Garnier J; Gaillardin C

AN 96367070 MEDLINE

L73 ANSWER 12 OF 108 MEDLINE DUPLICATE 2

TI Effects of signal peptide mutations on processing of **Bacillus**

stearothermophilus **alpha-amylase** in Escherichia coli.  
 SO MICROBIOLOGY, (1995 Mar) 141 ( Pt 3) 649-54.  
 Journal code: 9430468. ISSN: 1350-0872.  
 AU Suominen I; Meyer P; Tilgmann C; Glumoff T; Glumoff V; Kapyla J; Mantsala P  
 AN 95227363 MEDLINE

L73 ANSWER 13 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
 TI Bacillus licheniformis, Bacillus stearothermophilus and **Bacillus**  
 amyloliquefaciens **alpha-amylase** enzyme engineering  
 by site-directed mutagenesis;  
 DNA sequence; application in a surfactant or a starch liquefaction  
 composition  
 AN 1994-13784 BIOTECHDS  
 PI WO 9418314 18 Aug 1994

L73 ANSWER 14 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
 TI Lipase and **alpha-amylase variant** stabilized  
 against peroxidase system;  
 Humicola lanuginosa or Bacillus licheniformis enzyme stabilization by  
 enzyme engineering for use in a surfactant composition  
 AN 1994-11299 BIOTECHDS  
 PI WO 9414951 7 Jul 1994

L73 ANSWER 15 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
 TI **Mutant alpha-amylase** from **Bacillus**  
 sp. use as surfactant, dish washing agent and liquefaction agent;  
 Bacillus or Aspergillus spp. thermostable enzyme with increased  
 thermostability and activity at low pH produced by enzyme engineering  
 AN 1994-04189 BIOTECHDS  
 PI WO 9402597 3 Feb 1994

L73 ANSWER 16 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
 TI Saccharomycopsis fibuligera **alpha-amylase** or  
**Bacillus** macerans cyclomaltodextrin-glucanotransferase mutant  
 preparation;  
 enzyme engineering for improved production of oligosaccharide  
 AN 1995-00657 BIOTECHDS  
 PI JP 06253836 13 Sep 1994

L73 ANSWER 17 OF 108 MEDLINE DUPLICATE 3  
 TI Site-directed mutagenesis reveals critical importance of the catalytic  
 site in the binding of alpha-amylase by wheat proteinaceous inhibitor.  
 SO BIOCHEMISTRY, (1994 Jun 28) 33 (25) 7925-30.  
 Journal code: 0370623. ISSN: 0006-2960.  
 AU Takase K  
 AN 94281224 MEDLINE

L73 ANSWER 18 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 4  
 TI C-TERMINAL TRUNCATIONS OF A THERMOSTABLE **BACILLUS**  
**-STEAROTHERMOPHILUS ALPHA-AMYLASE**  
 SO PROTEIN ENGINEERING, (OCT 1994) Vol. 7, No. 10, pp. 1255-1259.  
 ISSN: 0269-2139.  
 AU VIHINEN M (Reprint); PELTONEN T; IITIA A; SUOMINEN I; MANTSALA P  
 AN 94:668008 SCISEARCH

L73 ANSWER 19 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISIDUPLICATE 5  
 TI CHANGES IN OPTIMUM PH AND THERMOSTABILITY OF **ALPHA-**  
**AMYLASE** FROM **BACILLUS**-LICHENIFORMIS BY SITE-DIRECTED  
 MUTAGENESIS OF HIS-235 AND ASP-328  
 SO BULLETIN OF THE KOREAN CHEMICAL SOCIETY, (20 OCT 1994) Vol. 15, No. 10,  
 pp. 832-835.  
 ISSN: 0253-2964.  
 AU KIM M S (Reprint); LEE S K; JUNG H S; YANG C H

AN 94:725048 SCISEARCH

L73 ANSWER 20 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
TI Experimental study on the technological conditions of **.alpha.-amylase** fermentation with **Bacillus mutant**  
SO Zhejiang Gongxueyuan Xuebao (1994), (2), 56-61  
CODEN: ZGXUEM; ISSN: 1000-209X  
AU Zheng, Yuguo; Cao, Xiaoru; Lu, Jianwei; Li, Xiaoqin  
AN 1994:653778 HCAPLUS  
DN 121:253778

L73 ANSWER 21 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI **Mutant Bacillus licheniformis alpha-amylase** promoter, vector and DNA sequence;  
application in alpha-amylase, cyclomaltodextrin-glucanotransferase,  
glucosyltransferase and protease production  
AN 1993-09548 BIOTECHDS  
PI WO 9310249 27 May 1993

L73 ANSWER 22 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI New **mutant Bacillus licheniformis alpha-amylase** signal peptide sequence;  
to give reduced processing ambiguity during e.g. human recombinant  
interleukin-3 protein secretion  
AN 1994-01346 BIOTECHDS  
PI EP 572088 1 Dec 1993

L73 ANSWER 23 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
TI Gene expressing in **Bacillus licheniformis** using especially **.alpha.-amylase promoter variant**  
SO PCT Int. Appl., 63 pp.  
CODEN: PIXXD2

IN Joergensen, Steen Troels; Joergensen, Per Linaa  
AN 1993:553380 HCAPLUS  
DN 119:153380

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9310248	A1	19930527	WO 1992-DK337	19921113
W: FI, JP, KR				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE				
JP 07503363	T2	19950413	JP 1993-508898	19921113
EP 672154	A1	19950920	EP 1992-923721	19921113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
FI 9402227	A	19940513	FI 1994-2227	19940513

L73 ANSWER 24 OF 108 Elsevier BIOBASE COPYRIGHT 2003 Elsevier Science B.V.  
DUPLICATE

AN 1994016204 ESBIIOBASE  
TI Crystallization and preliminary X-ray studies of wild type and  
catalytic-site **mutant alpha-amylase** from  
**Bacillus subtilis**  
AU Mizuno H.; Morimoto Y.; Tsukihara T.; Matsumoto T.; Takase K.  
CS H. Mizuno, Japan.  
SO Journal of Molecular Biology, (1993), 234/4 (1283-1293)  
CODEN: JMOBAK ISSN: 0022-2836  
DT Journal; Article  
LA English  
SL English

L73 ANSWER 25 OF 108 MEDLINE DUPLICATE 7  
TI Crystallization and preliminary X-ray studies of wild type and  
catalytic-site **mutant alpha-amylase** from  
**Bacillus subtilis**.  
SO JOURNAL OF MOLECULAR BIOLOGY, (1993 Dec 20) 234 (4) 1282-3.

Journal code: 2985088R. ISSN: 0022-2836.

AU Mizuno H; Morimoto Y; Tsukihara T; Matsumoto T; Takase K  
AN 94087744 MEDLINE

L73 ANSWER 26 OF 108 MEDLINE DUPLICATE 8  
TI Effect of mutation of an amino acid residue near the catalytic site on the  
activity of **Bacillus** stearothermophilus **alpha-**  
**amylase**.  
SO EUROPEAN JOURNAL OF BIOCHEMISTRY, (1993 Feb 1) 211 (3) 899-902.  
Journal code: 0107600. ISSN: 0014-2956.  
AU Takase K  
AN 93170327 MEDLINE

L73 ANSWER 27 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
DUPLICATE 9  
TI Transformation of **Bacillus subtilis** in **alpha-**  
**amylase** production by **mutant** DNA.  
SO Journal of the Chinese Agricultural Chemical Society, (1993) Vol. 31, No.  
4, pp. 454-465.  
ISSN: 0578-1736.  
AU Wang, Mei-Jen; Chou, Cheng-Chun; Yu, Roch-Chui  
AN 1994:269713 BIOSIS

L73 ANSWER 28 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI Purification and characterization of a thermostable **alpha-**  
**amylase** from **Bacillus licheniformis**;  
enzyme isolation and properties  
SO J.Biotechnol.; (1993) 28, 2-3, 277-89  
CODEN: JBITD4  
AU Ivanova V N; Dobрева E P; Emanuilova E I  
AN 1993-07233 BIOTECHDS

L73 ANSWER 29 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI Stability of industrial enzymes;  
enzyme stabilization by chemical modification or enzyme engineering  
(conference paper)  
SO Stud.Org.Chem.; (1993) 47, 111-31  
CODEN: 9999T  
AU Misset O  
AN 1994-05917 BIOTECHDS

L73 ANSWER 30 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
TI New thermostable forms of **Bacillus licheniformis alpha-**  
**-amylase**;  
enzyme engineering by specific amino acid substitutions at positions  
133 and or 209, for simultaneous gelation and liquefaction of starch,  
e.g. in brewing  
AN 1993-03609 BIOTECHDS  
PI FR 2676456 20 Nov 1992

L73 ANSWER 31 OF 108 HCAPLUS COPYRIGHT 2003 ACS DUPLICATE 11  
TI Thermostable **variants** of **Bacillus licheniformis**  
**alpha-amylase**, manufacture with transgenic cells, and  
their use  
SO Fr. Demande, 18 pp.  
CODEN: FRXXBL  
IN Declerck, Nathalie; Joyet, Philippe; Gaillardin, Claude  
AN 1993:55127 HCAPLUS  
DN 118:55127

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2665178	A1	19920131	FR 1990-9679	19900730

L73 ANSWER 32 OF 108 MEDLINE DUPLICATE 12

TI Hyperthermostable variants of a highly thermostable alpha-amylase.  
 SO BIO/TECHNOLOGY, (1992 Dec) 10 (12) 1579-83.  
 Journal code: 8309273. ISSN: 0733-222X.  
 AU Joyet P; Declerck N; Gaillardin C  
 AN 93168398 MEDLINE

L73 ANSWER 33 OF 108 MEDLINE DUPLICATE 13  
 TI Interaction of catalytic-site **mutants** of **Bacillus subtilis alpha-amylase** with substrates and acarbose.  
 SO BIOCHIMICA ET BIOPHYSICA ACTA, (1992 Aug 21) 1122 (3) 278-82.  
 Journal code: 0217513. ISSN: 0006-3002.  
 AU Takase K  
 AN 92369111 MEDLINE

L73 ANSWER 34 OF 108 MEDLINE DUPLICATE 14  
 TI Functional relationships between cyclodextrin glucanotransferase from an alkalophilic **Bacillus** and **alpha-amylases**.  
 Site-directed **mutagenesis** of the conserved two Asp and one Glu residues.  
 SO FEBS LETTERS, (1992 Jan 13) 296 (1) 37-40.  
 Journal code: 0155157. ISSN: 0014-5793.  
 AU Nakamura A; Haga K; Ogawa S; Kuwano K; Kimura K; Yamane K.  
 AN 92111781 MEDLINE

L73 ANSWER 35 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
 TI Comparison of microbial amylase production by various mutants derived from *Bacillus amyloliquefaciens*  
 SO Gongye Weishengwu (1992), 22(2), 26-9  
 CODEN: GOWEEK; ISSN: 1001-6678  
 AU Hu, Xuezhi; Hou, Qinfang; Ling, Chen  
 AN 1992:549367 HCAPLUS  
 DN 117:149367

L73 ANSWER 36 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
 TI A mutant enzyme with reduced stability;  
**Bacillus amyloliquefaciens alpha-amylase**  
**mutant** expression in e.g. *Escherichia coli*, *Bacillus*,  
*Aspergillus* spp.; bread improver with reduced thermostability during  
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L73 ANSWER 39 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
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L73 ANSWER 40 OF 108 LIFESCI COPYRIGHT 2003 CSA  
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L73 ANSWER 41 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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L73 ANSWER 42 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
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 PRODUCTION IN **BACILLUS**-LICHENIFORMIS  
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L73 ANSWER 43 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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 hyperproducing **Bacillus** sp. No. 32H417 and some properties of  
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 DN 119:156049

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PI	JP 02222687	A2	19900905	JP 1989-43561	19890225

L73 ANSWER 45 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
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L73 ANSWER 47 OF 108 MEDLINE DUPLICATE 17  
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L73 ANSWER 48 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
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L73 ANSWER 50 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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 AN 1990:175379 HCAPLUS  
 DN 112:175379

L73 ANSWER 51 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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L73 ANSWER 52 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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 CODEN: EPXXDW  
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 AN 1990:53755 HCAPLUS  
 DN 112:53755

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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	R: BE, DE, FR, GB, IT, NL				
	ES 2007758	A6	19890701	ES 1987-3510	19871207
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- L73 ANSWER 56 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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- L73 ANSWER 59 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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 DN 110:187342

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PI	EP 285123	A2	19881005	EP 1988-105163	19880330
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	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	FI 8801530	A	19881004	FI 1988-1530	19880331
	JP 01020089	A2	19890124	JP 1988-81159	19880401

L73 ANSWER 61 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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L73 ANSWER 64 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
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L73 ANSWER 65 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
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L73 ANSWER 66 OF 108 HCAPLUS COPYRIGHT 2003 ACS

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CODEN: SGXUED; ISSN: 1000-3061  
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L73 ANSWER 67 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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L73 ANSWER 68 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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L73 ANSWER 70 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
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L73 ANSWER 71 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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L73 ANSWER 72 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
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L73 ANSWER 73 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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 CODEN: PRENE9  
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L73 ANSWER 74 OF 108 SCISEARCH COPYRIGHT 2003 THOMSON ISI  
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L73 ANSWER 77 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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 created by site-directed mutagenesis (conference abstract)  
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L73 ANSWER 78 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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L73 ANSWER 79 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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L73 ANSWER 80 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
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 CODEN: SMHAEH.

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L73 ANSWER 81 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
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 CODEN: BIOHAO

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L73 ANSWER 82 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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 AN 1986:475540 HCAPLUS  
 DN 105:75540

L73 ANSWER 83 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.  
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 CODEN: BIOHAO; ISSN: 0006-307X

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L73 ANSWER 85 OF 108 MEDLINE DUPLICATE 27  
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L73 ANSWER 86 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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 CODEN: 54VYAW

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L73 ANSWER 87 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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CODEN: EPXXDW  
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AN 1984:3412 HCAPLUS  
DN 100:3412

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	EP 92235	A3	19840912		
	R: BE, CH, DE, FR, IT, LI, NL, SE				
	US 4465773	A	19840814	US 1982-370433	19820421
	AU 8313221	A1	19831027	AU 1983-13221	19830407
	AU 555540	B2	19861002		
	ZA 8302452	A	19831228	ZA 1983-2452	19830407
	IL 68378	A1	19860228	IL 1983-68378	19830414
	DK 8301733	A	19831022	DK 1983-1733	19830420
	GB 2118968	A1	19831109	GB 1983-10737	19830420
	GB 2118968	B2	19850717		
	JP 58190391	A2	19831107	JP 1983-69280	19830421

L73 ANSWER 88 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
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CODEN: EPXXDW  
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	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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=> d ab 9,11,14,17,26,32,35,44,45,47,52,62

L73 ANSWER 9 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI  
AB An amylolytic enzyme (I) derived from **Bacillus licheniformis**  
**alpha-amylase** (EC-3.2.1.1) (or an enzyme with 70%  
identity) is new, containing 1 or more amino acid changes at position 104  
(Asn to Asp), 128 (Val to Glu), 187 (Ser to Asp) and 188 (Asn to Asp) of  
the wild-type enzyme. Also claimed are: a nucleic acid encoding (I); a  
vector for the expression of (I); host cells expressing (I); and, a  
detergent composition containing (I). (I) preferably has an additional  
amino acid change, providing the enzyme with increased thermostability,  
preferably the mutations His 133 to Tyr 133 and Thr 149 to Ile 149. (I)  
may also have at least 1 amino acid change providing the enzyme with  
improved oxidation stability, preferably by changing a Met residue to  
another amino acid, e.g. Met 197. The mutant enzyme has higher activity  
under optimal and suboptimal conditions (pH less than 6.5 or over 7  
and/or Ca<sup>2+</sup> concentration under 50 ppm). (I) may be used for starch  
degradation (syrup, isosyrup or ethanol production), in textile or paper  
desizing, in the brewing industry and in household surfactant  
compositions. (32pp)

L73 ANSWER 11 OF 108 MEDLINE DUPLICATE 1  
AB We have identified previously two critical positions for the  
thermostability of the highly thermostable **alpha-amylase**  
from **Bacillus licheniformis**. We have now introduced all 19  
possible amino acid residues to these two positions, His133 and Ala209.  
The most favourable substitutions were to Ile and Val, respectively, which  
both increased the half-life of the enzyme at 80 degrees C by a factor of  
approximately 3. At both positions a stabilizing effect of hydrophobic  
residues was observed, although only in the case of position 133 could a  
clear correlation be drawn between the hydrophobicity of the inserted



amino acid and the gain in protein stability. The construction of double mutants showed a cumulative effect of the most favourable and/or deleterious substitutions. Computer modelling was used to generate a 3-D structure of the wild-type protein and to model substitutions at position 209, which lies in the conserved (alpha/beta)<sub>8</sub> barrel domain of alpha-amylase; Ala209 would be located at the beginning of the third helix of the barrel, in the bottom of a small cavity facing the fourth helix. The model suggests that replacement by, for example, a valine could fill this cavity and therefore increase intra- and interhelical compactness and hydrophobic interactions.

L73 ANSWER 14 OF 108 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI

AB A new lipase (EC-3.1.1.3) or **alpha-amylase** (EC-3.2.1.1) **variant**, stabilized towards inactivation caused by a peroxidase (EC-1.11.1.7) system (a peroxidase, an H<sub>2</sub>O<sub>2</sub> source and an enhancing agent) has at least 1 native Tyr residue deleted or substituted with Phe, Leu, Ile, Val, Gln, Asn, Ser, Thr, Glu or His. The lipase variant is from *Humicola lanuginosa* (preferred), *Humicola brevispora*, *Humicola brevis* var. *thermoidea*, *Humicola insolens*, *Pseudomonas cepacia*, *Pseudomonas fragi*, *Pseudomonas stutzeri*, *Pseudomonas fluorescens*, *Fusarium oxysporum*, *Rhizomucor miehei*, *Candida antarctica* or *Candida cylindracea*, and has a mutation at position 16, 21, 53, 138, 164, 171, 194, 213, 220 or 261. The **alpha-amylase variant** is from *Bacillus licheniformis* (preferred) or *Aspergillus* sp., and has a mutation at position 10, 14, 31, 46, 56, 59, 62, 77, 98, 150, 158, 175, 193, 195, 198, 203, 219, 262, 273, 290, 302, 348, 358, 363, 367, 394, 396, 402, 439 or 480. The enzymes are useful in surfactant compositions, and have improved compatibility with peroxidase systems used to inhibit dye transfer. (28pp)

L73 ANSWER 17 OF 108 MEDLINE DUPLICATE 3

AB A bacterial **alpha-amylase** from *Bacillus subtilis* was found to be strongly inhibited by wheat alpha-amylase inhibitors 0.53 and 0.19, which had previously been thought specific for animal alpha-amylase. Inhibition and gel filtration studies of site-directed **mutants** of *B. subtilis* **alpha-amylase** with the inhibitors indicated a direct correlation between the alpha-amylase activity and the inhibitory effect of inhibitor binding. A mutant enzyme His 180-->Asn, which was 20 times less active in terms of k<sub>cat</sub> than the wild type, was less sensitive to inhibition by similar degrees, while the specificity for 0.53 and 0.19 changed significantly as a result of the mutation. Catalytic-site mutants that were completely devoid of catalytic activity virtually lost the ability to bind inhibitors, even though they retained high affinities for substrates. The results show that the integrity of the catalytic site is crucial for inhibitor binding and, despite the previously observed tight binding, reveal a subtle nature of the interaction between alpha-amylase and the wheat inhibitor, which leads to a proposal of a two-step mechanism for the binding interaction.

L73 ANSWER 26 OF 108 MEDLINE DUPLICATE 8

AB Site-directed **mutagenesis** of a thermostable **alpha-amylase** from *Bacillus stearothermophilus* was performed to assess the role of amino acid residues near the catalytic site in catalysis. Asn329 is presumed to be adjacent to the proposed catalytic residue Asp331. Its mutation to Lys, which is found at the corresponding position in pullulanase, resulted in the loss of 99.7% of the activity, while the mutation to Asp or Val did not drastically reduce the activity. The mutation to Val altered the temperature/activity profile so that the activity was reduced to 25% of wild-type alpha-amylase at 60 degrees C but was over twofold greater at 5 degrees C. This effect could be ascribed to a decrease in the activation enthalpy by 32%. The mutation to Asp or Lys altered the pH/activity profile concomitant with possible changes in the ionization state of the groups introduced. These results show the

feasibility of altering and possibly improving the enzyme activity by mutagenesis of residues near the catalytic groups.

- L73 ANSWER 32 OF 108 MEDLINE DUPLICATE 12  
AB Genetic screening at temperatures between 70-80 degrees C far exceeds the range of growth of most bacteria, and is not applicable to isolate easily thermostable protein variants. We describe a temperature shift protocol and an in vivo screening method which allowed us to identify a hyperthermostable **variant** of the thermostable **alpha-amylase** from **Bacillus licheniformis**. Our strategy was to select, after hydroxylamine mutagenesis, an intragenic suppressor mutation which overcomes a mutation leading to a thermolabile enzyme. Sequence analysis of the mutated gene revealed only one change in the amino acid sequence, substituting a valine for alanine at position 209. This single amino acid replacement increased the half-life of the protein at 90 degrees C by a factor of two to three relative to the wild-type enzyme. When this substitution was combined with another stabilizing substitution (H133Y) we described previously, the stabilizing effects were additive. The half-life of the new protein was about 12 hours at 90 degrees C, corresponding to a nine to ten-fold increase over the wild-type enzyme and the industrial **Bacillus licheniformis alpha-amylase Termamyl**. These mutations are located in a predicted folding domain of the protein which appears crucial in determining thermal stability.
- L73 ANSWER 35 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
AB Several microorganism **mutants** which produced **.alpha.-amylase** were isolated from the parent strain BF7658. The strains produced amylase as well as alk. proteinase. Optimal pH was 6. Ca2+ at >0.6% suppressed enzyme prodn.
- L73 ANSWER 44 OF 108 HCAPLUS COPYRIGHT 2003 ACS  
AB B. subtilis .alpha.-amylase (I) C-terminal deletion mutants having higher sp. activity than wild type I are given. These I mutants have enzymic activity in broader pH range than the wild type I. The deletion (123-468 bp) is made at 3' end of the codon for Lys480 of I. I gene was cloned from B. subtilis and used to prep. expression plasmid pTC237-76 from which 3 expression plasmids encoding deletion mutants were prepd. Into B. subtilis plasmid pTB53, the wild I gene and mutant genes were subcloned and transformed into B. subtilis. B. subtilis transformants produced I and its deletion mutants by aerobic fermm. The deletion mutants of I had sp. activities of 624, 582, and 609 unit/mg, resp.; the wild type I had sp. activity of 223 unit/mg. Moreover, the deletion mutants of I had optimal pH at 5.5-7.5, as compared to 5.5-6.5 for that of the wild type I.
- L73 ANSWER 45 OF 108 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
- L73 ANSWER 47 OF 108 MEDLINE DUPLICATE 17  
AB The relationship between structure, activity, and stability of the thermostable **Bacillus stearothermophilus alpha-amylase** was studied by site-directed mutagenesis of the three most conserved residues. Mutation of His-238 to Asp involved in Ca2+ and substrate binding reduced the specific activity and thermal stability, but did not affect the pH and temperature optima. Replacement of Asp-331 by Glu in the active site caused almost total inactivation. Interestingly, in prolonged incubation this mutant enzyme showed an altered end-product profile by liberating only maltose and maltotriose. Conservative mutation of the conserved Arg-232 by Lys, for which no function has yet been proposed, resulted in lowered specific activity: around 12% of the parental enzyme. This mutant enzyme had a wider pH range but about the same temperature optimum and thermal stability as the wild-type enzyme. Results obtained with different mutants were interpreted by computer aided molecular modeling.

L73 ANSWER 52 OF 108 HCAPLUS COPYRIGHT 2003 ACS

AB The title enzyme (I) is manufd. by culturing Bacillus NCIB 12563 at 50-70.degree., preferably 55-65.degree., and recovering I from the culture medium. Bacillus NCIB 12563, an over-prodn. mutant of parental strain 11886, was shake-cultured in medium contg. corn steep liquor, NH4Cl, sol. starch, and yeast ext. at 56.degree. for 24 h to produce I 917 units/mL compared to 216 for that of the parental strain 11886. I was isolated from the culture medium by fractionation with acetone (4.degree.). Prodn. of I on an industrial scale was given.

L73 ANSWER 62 OF 108 MEDLINE .DUPLICATE 21

AB The oligonucleotide encoding Bam HI recognition site having the structure pCGGGATC had been inserted into the recognition sites MspI of the B. amyloliquefaciens alpha-amylase gene, which was cloned in pTG29B plasmid. The alpha-amylase gene had no BamHI sites before mutagenesis. The set of pNSBamHI plasmids with BamHI site at four different positions was obtained. It was shown that all the **mutant alpha-amylases** possess different specific activities. One of the mutant proteins possesses reduced thermostability. The **mutant alpha-amylases** can be used for further experiments on protein-engineering of liquefying-type alpha-amylases.

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